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Complete Medium Prepared with PRIME-XV MSC XSFM Dual Component Kit

Stable at Room Temperature for Up to 9 Days

APPLICATION NOTE

INTRODUCTION

This application note provides data on the functionality and chemical stability of the combined components of the PRIME-XV MSC XSFM Dual Component Kit with Phenol Red. The complete medium, prepared by combining the base and supplement, was kept for up to 9 days at room temperature and protected from light. No significant difference was observed in the chemical composition or functionality between the complete media kept at 2–8°C and complete media kept at room temperature (20–23°C), protected from light.

The large-scale expansion and manufacture of mesenchymal stromal/stem cells (MSCs) faces numerous scale-up difficulties. Due to the culture surface area requirement for adherent cell cultures, traditional stirred-tank bioreactor systems are ill-equipped to address their culture needs without the aid of microcarriers, which introduce an additional level of complexity to cell expansion and downstream applications.

The Quantum hollow fiber bioreactor system from Terumo Blood and Cell Technologies (BCT) provides a large surface area for MSC adhesion and expansion, as well as a highly customizable expansion program that can be modified to accommodate the metabolic needs of MSCs derived from various sources. Though the Quantum provides a temperature-controlled environment for cell expansion, the cell culture media used for the week-long expansion protocol is left to hang above the instrument at room temperature, protected from light. This extended exposure of complete cell culture media to suboptimal storage temperatures raises understandable concerns regarding the stability of the media and its functionality for the duration of the culture.

In this experiment, PRIME-XV MSC XSFM Base with Phenol Red was combined with PRIME-XV MSC XSFM Supplement and the complete medium was stored at room temperature, protected from light, for up to 9 days prior to initiating MSC expansion in 6-well cell culture plates. Results of this study demonstrate that media stored for 9 days at room temperature did not suffer significant decrease in function over 3 passages compared to the properly stored control, indicating that the PRIME-XV media should remain fully functional throughout the Quantum MSC expansion protocol.

MATERIALS

Reagents		Disposables	Equipment
PRIME-XV MSC XSFM Dual Component Kit with Phenol Red (FUJFILM Biosciences, Catalog # 91149DC) consisting of: • **PRIME-XV MSC XSFM Base (+) Phenol Red**, (FUJIFILM Biosciences, Catalog # 91235-5 L) • **PRIME-XV MSC XSFM Dual Component Supplement**, (FUJIFILM Biosciences, Catalog # 91236-10 mL	• **Recombinant Human Fibronectin**, (R&D Systems, Catalog # 4305-FBN) • **TrypLE Express Enzyme**, (Thermo Fisher, Catalog # 12604021)	• **CellBIND 6-well Plates,** (Corning, Catalog # 3335) • **T-225 Tissue Culture Flasks**, (Thermo Fisher, Catalog # 130330)	• **Vi-CELL XR Cell Viability Analyzer** (Beckman Coulter) • **Cellaca MX High-throughput Cell Counter** (Revvity) • **FACSymphony A3 Cell Analyzer** (BD Bioscience)

METHODS

Media preparation

PRIME-XV MSC XSFM Base (+) Phenol Red was combined with PRIME-XV MSC XSFM Dual Component Supplement according to the product insert. Combined media were aseptically aliquoted into 6 x 250 mL sterile PET bottles. Bottles were covered with aluminum foil to protect from light.

One bottle was placed in a protected, dark environment at room temperature. The rest were refrigerated at 2–8°C. One additional bottle was removed every other day and stored at room temperature. Experiment was initiated on the day after the fifth bottle is removed, using the sixth bottle as a fresh control. All media bottles were subsequently refrigerated at 2–8°C for the rest of the experiment.

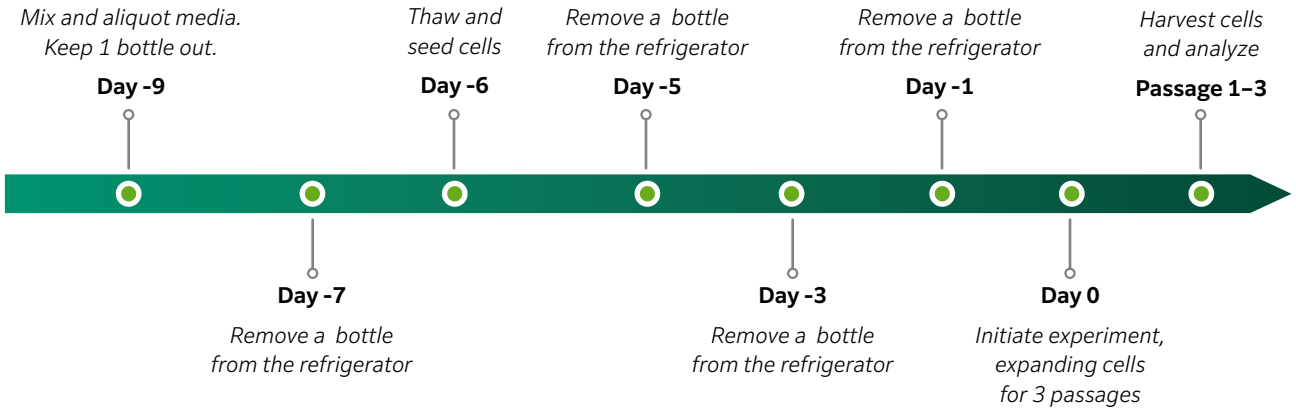

Plate cells

MSCs were thawed into fibronectin-coated T-225 tissue culture flasks 5–6 days before initiating the experiment. They were expanded for one passage to allow for post-cryopreservation recovery. After the passage, MSCs were harvested from the T-225 flasks and seeded at 2 x 10^3 cells/cm^2 into 6-well CellBIND plates in triplicate for each of the six media conditions. The cells were expanded for three passages and fed with complete media replacement every 2–3 days as needed. Once cells reached ~80–90% confluence, they were passaged.

Harvest and analyze cells

Cells were harvested once they reached 80–90% confluence on the third passage in the 6-well plates. The cells were counted and stained the MSC surface markers, as defined by the Mesenchymal Stromal Cell Committee of the International Society for Cell & Gene Therapy (ISCT).

RESULTS

Bone marrow (BM) and umbilical cord (UC) derived MSCs successfully expand in complete PRIME-XV MSC XSFM Dual Component media kept at room temperature for up to 9 days **(Figure 1)**. Population doubling level (PDL) shows no significant difference in expansion between conditions cultured in media that had been kept at room temperature for 0 to 9 days **(Figure 1A)**. Cell viability remains above 85% for the BMMSC s over the 3 passages, with UCMSCs maintaining an average viability at or above 80% **(Figure 1B).**

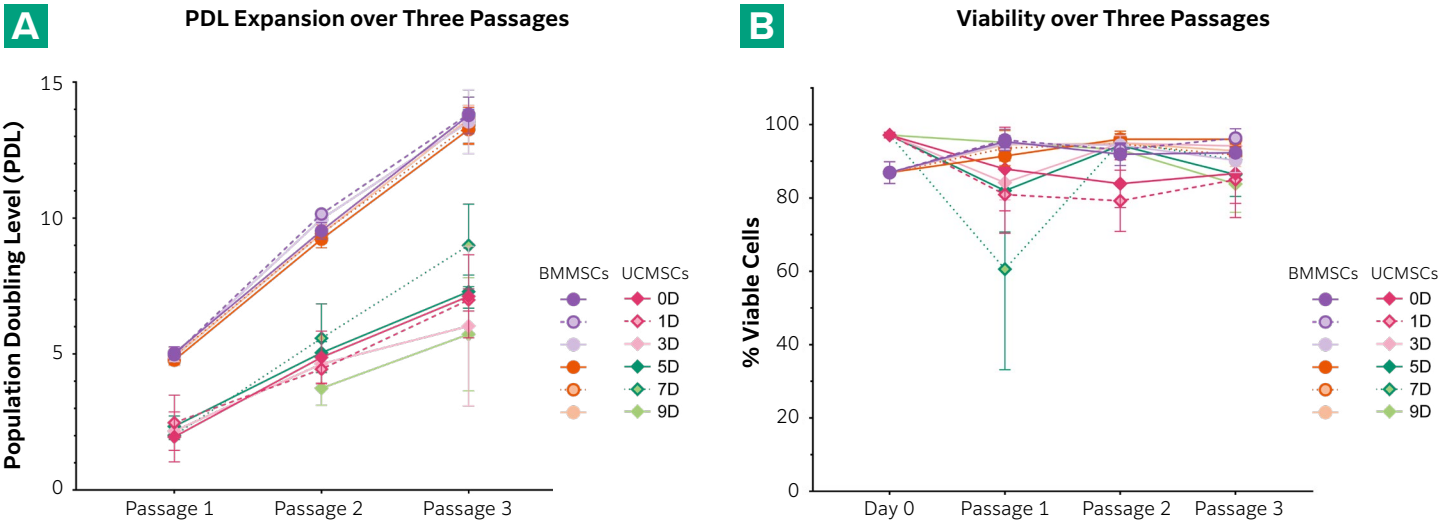

Figure 1. Expansion of BMMSCs and UCMSCs with complete PRIME-XV MSC XSFM Dual Component media kept at room temperature for up to 9 days. PDL **(A)** and cell viability **(B)** for BMMSCs and UCMSCs. PDL = log (Cf/Ci), where Cf is the total cell count at collection and Ci is the total cell count at seeding, calculated at the endpoint of each of 3 passages. Results represent triplicate wells per experimental condition per donor.

Expanded MSCs express MSC surface markers CD73 and CD90 after 3 passages **(Figure 2)**. CD34 and CD45 expression was absent throughout all passages (data not shown). CD73 and CD90 levels were maintained above 90% in BMMSCs **(Figure 2A)** and 80% in UCMSCs **(Figure 2B)** for all 3 passages. CD105 levels fluctuated with both donors, starting at 35–45% on Day 0, increasing at Passage 1, and decreasing over subsequent passages. Expression of surface markers was not affected by media exposure to room temperature storage.

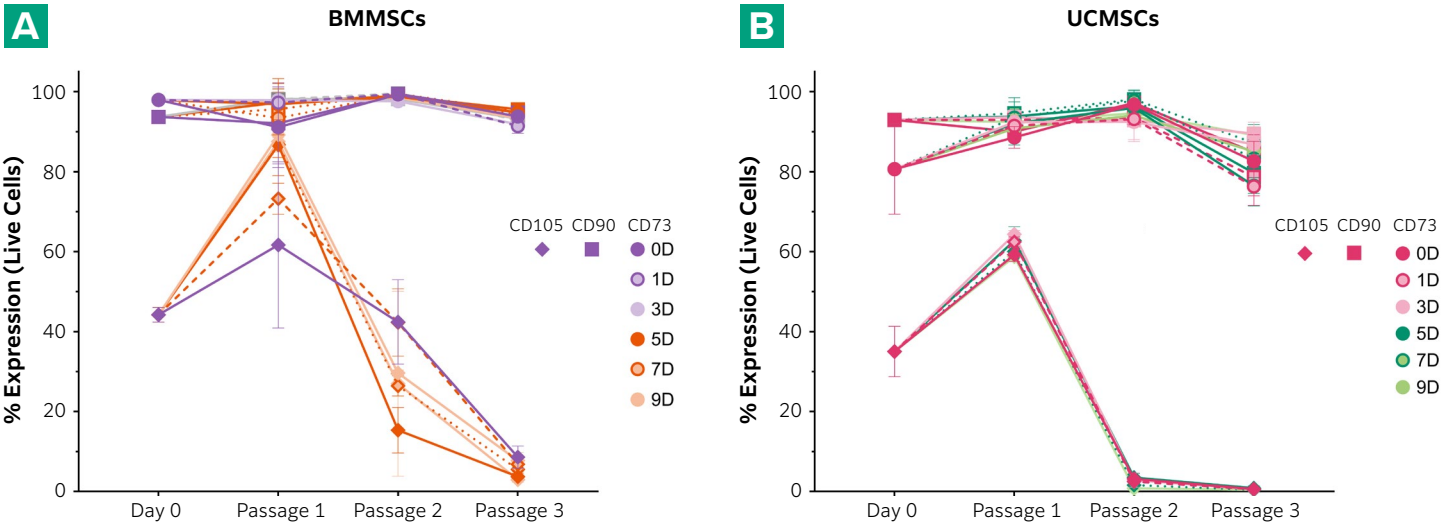

Figure 2. MSC surface markers assessed after 3 passages. BMMSCs **(A)** and UCMSCs **(B)** were stained with fluorescent antibodies against CD34, CD45, CD73, CD90, and CD105 at the end of each passage, alongside isotype-controlled samples and negative controls that were used to gate on positive populations. Data represent triplicate wells per experimental condition per donor. The results were collected on the flow cytometer FACSymphony A3 and analyzed using the software FlowJo.

CONCLUSION

Complete medium prepared by combining the components of the PRIME-XV MSC XSFM Dual Component Kit with Phenol Red, consisting of the base and supplement, maintains stability and full functionality for up to 9 days of storage at room temperature, while protected from light. Though exact results will differ depending on the donor-to-donor variation, complete medium prepared with the PRIME-XV MSC XSFM Dual Component Kit with Phenol Red may be used for MSC expansion in Quantum bioreactors, or other bioreactors requiring room temperature media storage for up to 9 days, without concern for media function declining over up to 9 days of culture. These conclusions are likewise applicable to complete medium prepared with the PRIME-XV MSC XSFM Dual Component Kit without Phenol Red, as the only difference in formulation is the absence of phenol red in the base component.

Customers can use PRIME-XV MSC XSFM Dual Component Kit with or without Phenol Red for MSC expansion in culture conditions requiring extended room temperature media storage with confidence, as long as the medium is kept sterile and protected from light.

FUJIFILM
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fujifilmbiosciences.fujifilm.com

FUJIFILM Biosciences – Corporate
2501 Pullman St., Santa Ana, Suite 200,
CA 92705 USA
Phone: 1 (949) 261-7800
Toll Free: 1 (800) 437-5706
Fax: 1 (949) 261-6522
Support: fisitmrequest@fujifilm.com